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NATURAL HEMOLYSINS IN HUMAN SERUM *

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In conducting complement fixation work with human serum, we have commonly used the sheep hemolytic system, altho we knew its imperfection, namely, the presence of variable amounts of natural antisheep hemolysin in human serum. It is important, on account of quantitative factors, to use a hemolytic system which is free from this source of error, that is, the presence of variable amounts of the same hemolysin in the human serum as that used in the hemolytic system.

We are aware that many other hemolytic systems have been advocated, such as antihuman, antiox, and antihen, but it was our object to determine more definitely which were the more advantageous from this standpoint. Because of the infrequent occurrence of isohemolysins, the antihuman hemolytic system is the ideal one, except that it is somewhat difficult to prepare in rabbits an antihuman amboceptor of sufficiently high titer to avoid the influence of hemagglutinins.

In the Bauer and Hecht-Weinberg modifications of the Wassermann reaction, the natural antisheep amboceptor is utilized and an immune amboceptor is not added. While in some sera a proper dose of hemolytic amboceptor is already present, this factor is variable. Of course, in the presence of a large excess of amboceptor in a serum, hemolysis may be carried too far in any series of tubes containing that serum, in cases of only slight fixation of complement. In other words, with larger amounts of hemolytic amboceptor, smaller amounts of complement are necessary for complete hemolysis and these quantitative factors are of importance in complement fixation reactions. While, however, the importance of natural hemolysins must not be overestimated in view of the common presence of natural antisheep hemolysin in human serum and the widespread use and general satisfaction of an antisheep hemolytic system in the Wassermann syphilis and other complement fixation tests, it may be important on occasions and for certain work to use a hemolytic system in which quantitative relations are not disturbed by the presence of natural hemolysins. This may

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be accomplished by removing the natural hemolysin or by adopting a hemolytic system free from this error.

While the literature contains numerous references to the presence of natural antisheep hemolysin in human serum, we were unable to find any literature bearing upon the question of natural hemolysins for the erythrocytes of other vertebrates since the publication of the summary made by Sacks,[1] which is incomplete and indefinite and a compilation of data gathered from different sources.

With the necessity for definite and accurate information in our own work we have examined a large number of human sera for natural hemolysins for the erythrocytes of the sheep, dog, ox, goat, hog, rat, chicken, horse, rabbit, and guinea-pig by a method of titration which was used in a similar study of natural hemolysins in rabbit serum by Kolmer and Williams.[2]

PREPARATION OF MATERIALS

Before testing, the sera were inactivated by heating to 55 C. for thirty minutes, as in preparation of sera for the Wassermann reaction. This inactivated serum was then taken and diluted with nine parts of salt solution (0.85 percent) and used in the following amounts: 0.05 c.c., 0.2 c.c., 0.4 c.c., 0.8 c.c., 1.0 c.c., and 2 c.c., corresponding respectively to 0.005, 0.02, 0.04, 0.08, 0.1, and 0.2 c.c. of the undiluted serum. Complement was furnished by fresh guinea-pig serum, using 1 c.c. of a 5 percent dilution in normal saline. The corpuscles were used in dose of 1 c.c. of a 2.5 percent suspension in all the tests, with the exception of the rat cells, which were in 2 percent suspension.

TECHNIC

In Tube 1 were placed 0.05 c.c. of the 1:10 inactivated human serum; in Tube 2, 0.2 c.c.; in Tube 3, 0.04 c.c.; in Tube 4, 0.8 c.c.; in Tube 5, 1 c.c.; and in Tube 6, 2 c.c. Then, 1 c.c. of the guinea-pig complement and 1 c.c. of the corpuscle suspension and enough normal salt solution were added to make the total volume 4 c.c. After shaking, the tubes were placed in the incubator at 37 C. for two hours, removed, and allowed to stand in the refrigerator over night, and the results read in the morning.

CONTROLS

Natural amboceptor was not tested for in complement, but was removed from the guinea-pig serum by mixing it with the same kind of corpuscles used in testing for natural hemolysin and allowing absorption to take place in the refrigerator. Each serum was tested in maximum dosage with the corpuscles to make sure that all native complement was rendered inactive. With several sets of reactions, specific immune hemolysins were used with the sera to make certain that none of the sera was anticomplementary. The corpuscle suspensions were always prepared of fresh blood and controlled as to undue fragility by suspending a dose in salt solution.

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[1] Kolle and Wassermann: Handbuch der pathogenen Microorganism, 1913, 2, p. 793.

[2] Jour. Infect. Dis., 1913, 13, p. 96.

Natural Antisheep Hemolysin.—Table 1 shows that inactivated human serum contains considerable antisheep hemolysin, only 7 percent of sera examined being free from it. This fact is generally recognized and is the reason for many of the modifications of the Wassermann reaction.

TABLE 1

RESULTS OF EXAMINATION OF 150 HUMAN SERA FOR ANTISHEEP HEMOLYSIN

Amount of Serum c.c.	100 % Hemolysis	75 % Hemolysis	25 % Hemolysis	No Hemolysis Percentage
0.005	1	9	24	65
0.02	25	10	28	37
0.04	40	12	20	28
0.08	53	14	14	19
0.1	58	18	7	17
0.2	64	20	9	7

Natural Antidog Hemolysin.—Table 2 shows that natural antidog hemolytic amboceptors are fairly common in human serum, being present in some degree in 82 percent of the sera examined.

TABLE 2

RESULTS OF EXAMINATION OF 25 HUMAN SERA FOR ANTIDOG HEMOLYSIN

Amount of Serum c.c.	100 % Hemolysis	75 % Hemolysis	25 % Hemolysis	No Hemolysis Percentage
0.005	0	0	0	100
0.02	0	0	0	100
0.04	0	0	0	100
0.08	0	0	12	88
0.1	0	24	36	40
0.2	16	36	30	18

Natural Antiox Hemolysin.—The amount of antiox hemolytic amboceptor was much less than that of the antidog and antisheep amboceptor and on account of the comparative ease of obtaining ox blood in abattoirs and preparing highly potent immune antiox hemolysins, an ox hemolytic system is used in several laboratories and has been recommended for routine work.

TABLE 3

RESULTS OF EXAMINATION OF 85 SERA FOR ANTIOX HEMOLYSIN

Amount of Serum c.c.	100 % Hemolysis	75 % Hemolysis	25 % Hemolysis	No Hemolysis Percentage
0.005	0	0	0	100
0.02	0	0	0	100
0.04	0	0	0	100
0.08	0	0	8	92
0.1	0	8	16	76
0.2	6	20	24	50

Natural Antigoat Hemolysin.—Of the twenty-five human sera examined for antigoat hemolysin, none showed any hemolysis in dosage less than 0.1 c.c., and none gave complete hemolysis with any of the doses used.

TABLE 4

RESULTS OF EXAMINATION OF 25 HUMAN SERA FOR ANTIGOAT HEMOLYSIN

Amount of Serum c.c.	100 % Hemolysis	75 % Hemolysis	25 % Hemolysis	Percentage No Hemolysis
0.005	0	0	0	100
0.02	0	0	0	100
0.04	0	0	0	100
0.08	0	0	0	100
0.1	0	0	4	96
0.2	0	8	16	76

Natural Antihog Hemolysin.—Human sera rarely contain natural antihog amboceptor, only three sera exhibiting it, giving more than 25 percent hemolysis in only one case of 100 sera examined.

TABLE 5

RESULTS OF EXAMINATION OF 100 SERA FOR ANTIHOG HEMOLYSIN

Amount of Serum c.c.	100 % Hemolysis	75 % Hemolysis	25 % Hemolysis	No Hemolysis Percentage
0.005	0	0	0	100
0.02	0	0	0	100
0.04	0	0	0	100
0.08	0	0	0	100
0.1	0	0	1	99
0.2	0	1	3	96

Natural Antirat Hemolysin.—When twenty-five sera were tested for hemolysis of the blood-cells of Mus norvegicus, in three cases 0.2 c.c. of serum gave 25 percent hemolysis, and so it may be deduced that human serum rarely contains this amboceptor.

TABLE 6

RESULTS OF EXAMINATION OF 25 SERA FOR HEMOLYSIS OF BLOOD-CELLS OF MUS NORVEGICUS

Amount of Serum c.c.	100 % Hemolysis	75 % Hemolysis	25 % Hemolysis	No Hemolysis Percentage
0.005	0	0	0	100
0.02	0	0	0	100
0.04	0	0	0	100
0.08	0	0	0	100
0.1	0	0	0	100
0.2	0	0	12	88

Natural Antichicken Hemolysin.—When twenty-five sera were tested for antichicken hemolytic amboceptors, only two cases exhibited any hemolysin, and then only 25 percent of the corpuscles were hemolyzed, even with 0.2 c.c. of serum.

TABLE 7

RESULTS OF EXAMINATION OF 25 SERA FOR ANTICHICKEN HEMOLYTIC AMBOCEPTOR

Amount of Serum c.c.	100 % Hemolysis	75 % Hemolysis	25 % Hemolysis	No Hemolysis Percentage
0.005	0	0	0	100
0.02	0	0	0	100
0.04	0	0	0	100
0.08	0	0	0	100
0.1	0	0	0	100
0.2	0	0	8	92

Natural Antihorse Hemolysin.—Of the twenty-five sera tested for antihorse amboceptor, 0.2 c.c. of serum gave 25 percent hemolysis with only one serum.

TABLE 8

RESULTS OF TESTS OF 25 SERA FOR ANTIHORSE AMBOCEPTOR

Amount of Serum c.c.	100 % Hemolysis	75 % Hemolysis	25 % Hemolysis	No Hemolysis Percentage
0.005	0	0	0	100
0.02	0	0	0	100
0.04	0	0	0	100
0.08	0	0	0	100
0.1	0	0	0	100
0.2	0	0	4	96

Natural Antirabbit Hemolysin.—The results of tests of twenty-five human sera against rabbit erythrocytes were the same as those for antihorse hemolysin.

TABLE 9

RESULTS OF TESTS OF 25 SERA AGAINST RABBIT ERYTHROCYTES

Amount of Serum c.c.	100 % Hemolysis	75 % Hemolysis	25 % Hemolysis	No Hemolysis Percentage
0.005	0	0	0	100
0.02	0	0	0	100
0.04	0	0	0	100
0.08	0	0	0	100
0.1	0	0	0	100
0.2	0	0	4	96

Natural Antiguinea-Pig Hemolysin.—Table 10 shows the results of the examination of fifty sera for antiguinea-pig hemolysin.

TABLE 10

Results of Examination of 50 Sera for Antiguinea-Pig Hemolysin

Amount of Serum c.c.	100 % Hemolysis	75 % Hemolysis	25 % Hemolysis	No Hemolysis Percentage
0.005	0	0	0	100
0.02	0	0	0	100
0.04	0	0	0	100
0.08	0	0	0	100
0.1	0	0	0	100
0.2	0	0	2	98

In this table we note that only one serum caused any hemolysis and, in that case, it was only partial: 25 percent with the 0.2 c.c. dose of serum. Owing to the fact that guinea-pig complement serum is used almost exclusively in hemolytic work it would appear that a guinea-pig hemolytic system should prove quite satisfactory because both complement serum and corpuscles are readily accessible. It is somewhat difficult, however, to produce a satisfactory rabbit immune hemolysin for guinea-pig cells, and this reduces the value of these erythrocytes in a hemolytic system.

Tables 1-10 give all the essential data, but it will be of greater interest to analyze the results more minutely. Tables 11 and 12 show the smallest amounts of human serum causing 100, 75, and 25 percent destruction of the red blood-cells of the various animals investigated and what percentage they bear to the number of sera tested.

TABLE 11

Showing the Smallest Amounts of Human Serum Giving 100, 75, and 25 Percent Hemolysis and Percentage of Cases in Which It May Be Expected

Blood-Cells	100% Hemolysis	75% Hemolysis	25% Hemolysis
Sheep	0.005 (1%)	0.005 (10%)	0.005 (34%)
Dog	0.2 (16%)	0.1 (24%)	0.08 (12%)
Ox	0.2 (6%)	0.1 (8%)	0.08 (8%)
Goat	....	0.2 (8%)	0.1 (4%)
Hog	....	0.2 (1%)	0.1 (1%)
Rat	....	....	0.2 (12%)
Chicken	....	....	0.2 (8%)
Horse	....	....	0.2 (4%)
Rabbit	....	....	0.2 (4%)
Guinea-pig	....	....	0.2 (2%)

In Table 12 we have summarized the presence of natural hemolysins in 0.2 c.c. of human serum.

TABLE 12

SUMMARY OF NATURAL HEMOLYSINS IN HUMAN SERUM (0.2 C.C. AMOUNTS)

Blood-Cells	No. of Sera Tested	Percentage Showing 100% Hemolysis	Percentage Showing 75% Hemolysis	Percentage Showing 25% Hemolysis	Percentage Showing No Hemolysis
Sheep	125	64	20	9	7
Dog	25	16	36	30	18
Ox	85	6	20	24	50
Goat	25	0	8	16	76
Hog	100	0	1	3	96
Rat	25	0	0	12	88
Chicken	25	0	0	8	92
Horse	25	0	0	4	96
Rabbit	25	0	0	4	96
Guinea-pig	50	0	0	2	98

The obvious conclusion from Tables 11 and 12 is that the sheep and dog are not the ideal hemolytic systems for performing complement fixation work with human serum, but that hog, rat, chicken, horse, rabbit, or guinea-pig systems are preferable, depending from the practical side upon the ease with which we can obtain these corpuscles and their specific amboceptors.